Universidade de Lisboa

Faculdade de Medicina Dentária

Insights on the Thermal Aging of Acrylic Reline Resins

Filipe Gaudêncio Pacheco

Dissertação

Mestrado Integrado em Medicina Dentária

2016

Universidade de Lisboa
Faculdade de Medicina Dentária

Insights on the Thermal Aging of Acrylic Reline Resins

Filipe Gaudêncio Pacheco

Dissertação orientada pela Professora Doutora Maria Cristina Bettencourt Neves e coorientada pela Professora Doutora Ana Francisca Bettencourt

Mestrado Integrado em Medicina Dentária

2016

TABLE OF CONTENTS

AGRADECIMENTOS

A produção deste trabalho contou com a colaboração de várias pessoas. a quem quero deixar os meus sinceros agradecimentos

À minha orientadora Professora Doutora Maria Cristina Bettencourt Neves, Professora Auxiliar da disciplina de Prostodontia Removível da Faculdade de Medicina Dentária da Universidade de Lisboa a quem quero agradecer por toda a confiança, amizade, orientação científica, disponibilidade e incentivo ao longo da elaboração desta tese de Mestrado. Tem sido sem dúvida uma grande referência pelo seu enorme sucesso profissional e qualidades humanas que muito valorizo.

À minha co-orientadora Professora Doutora Ana Francisca Bettencourt, Professora Auxiliar da Unidade Curricular de Dispositivos Médicos e Física do Departamento de Ciências Toxicológicas e Bromatológicas da Faculdade de Farmácia pelos ensinamentos e acompanhamento na realização do trabalho experimental e pela disponibilidade, apoio e incentivo com que sempre me orientou.

À minha colega Fábia Alexandre por toda a sua ajuda, tempo, simpatia e companheirismo ao longo deste projecto.

Gostaria também de agradecer aos meus amigos e colegas de turma que muito contribuíram para a minha alegria e crescimento pessoal ao longo destes últimos anos em especial à Tiffany Pinto, Mara Ramos, Carolina de Sá, Pedro Gomes, Sara Mendes, Isabel Rodrigues e Catarina Serra por todos os momentos fantásticos e pela verdadeira amizade que me têm dedicado.

Ao João Pedro Rosa que foi sem dúvida das melhores pessoas que surgiram na minha vida. O seu companheirismo, carinho, atenção e dedicação revelaram-se fundamentais para a minha felicidade

À Isabel Lucas por ter sido a pessoa que mais me acompanhou durante toda esta caminhada. A sua amizade, carinho e dedicação foram absolutamente fundamentais para a minha felicidade e crescimento pessoal. É, sem dúvida uma pessoa que representa um exemplo perfeito de combinação entre excelentes valores humanos com um grande sucesso académico e profissional.

Ao Duarte Afonso que muito admiro pela extraordinária pessoa que é. Não podia deixar de agradecer pela amizade e apoio absolutamente incondicional com que me tem presenteado nestes últimos anos. A sua coragem, espirito de sacrifício, dedicação e bondade serão para sempre uma grande referência na minha vida.

Aos docentes, alunos e funcionários da Faculdade de Medicina Dentária da Universidade de Lisboa que contribuíram para a minha formação pessoal e profissional.

À minha família, em especial aos meus pais pelo seu amor incondicional, respeito, apoio, carinho e confiança que depositaram em mim desde sempre. São a minha principal fonte de força e eles devo grande parte do meu sucesso e felicidade.

RESUMO

Apesar dos métodos e campanhas de prevenção aplicados no sentido de melhorar a saúde oral, a perda de peças dentárias ainda é um problema prevalente numa população significativa de idosos. Assim sendo, e tendo em conta o envelhecimento da população prevê-se um aumento na utilização de próteses totais ou parciais por um elevado número de pacientes no futuro.

As resinas acrílicas de rebasamento são materiais utilizados de forma frequente em medicina dentária. A sua principal função é a adaptação das próteses removíveis a alterações causadas nos tecidos de suporte após a perda de dentes.

A estomatite protética é uma condição recorrentemente observada em portadores de próteses removíveis. Esta patologia tem múltiplas causas (trauma, xerostomia, uso continuo da prótese) mas associa-se principalmente à infecção por espécies *Candida*, principalmente *Candida albicans* cuja capacidade de aderir a células hospedeiras ou a polímeros como as resinas acrílicas é uma característica essencial para a patogénese da infecção.

Atualmente, a terapêutica da estomatite protética é feita principalmente pela aplicação de fármacos através de um regime periódico por via tópica ou sistémica. Este método tem-se mostrado pouco eficaz principalmente devido a problemas de adesão à terapêutica por parte dos pacientes e à dificuldade em manter a dose necessária no local afectado por longos períodos de tempo. Desta forma foi necessária investigação no sentido de encontrar novas modalidades de tratamento.

Recentemente foi proposta a utilização de agentes de libertação controlada para o tratamento da estomatite protética. Esta intervenção terapêutica baseia-se na incorporação de fármacos em materiais como as resinas acrílicas com o objectivo de serem libertados de forma lenta e controlada. Os estudos indicam que estas formulações têm a capacidade de manter níveis terapêuticos no local pretendido muitas vezes superiores à concentração mínima inibitória das espécies alvo e por longos períodos de tempo. Adicionalmente, estes veículos são praticamente independentes da adesão à terapêutica do paciente e estão associados a menor interacção medicamentosa e efeitos colaterais. Apesar de vários fármacos terem sido incorporados em resinas acrílicas, a clorexidina tem mostrado os resultados mais promissores. A evidência tem demonstrado

que este antisséptico reduz a capacidade de adesão da C. *albicans* à superfície das próteses dentárias (processo essencial para a formação de biofilme) e não causa o aparecimento de estripes resistentes.

No entanto, a literatura carece de estudos que avaliem as alterações físicas provocadas nas resinas acrílicas aquando da sua incorporação com clorexidina e submetida a processos de envelhecimento.

Assim sendo, os objectivos do presente estudo foram o de avaliar a estabilidade de cor e variação de massa de duas resinas acrílicas de rebasamento directo (Ufi Gel Hard e Kooliner) e uma de rebasamento indirecto (Probase Cold) com diferentes concentrações de clorexidina incorporada, após submetidas a termociclagem, e o estudo posterior de libertação do fármaco destas resinas ao longo de 28 dias.

Para cada um dos materiais foram produzidos 5 grupos de espécimes, um de controlo e 4 com diferentes concentrações de clorexidina incorporada (2.5%, 5%, 7.5% e 10%). Foram avaliados um total de 45 espécimes em forma de cilindro (12mm de comprimento por 6mm de diâmetro). Os espécimes foram pesados numa balança de precisão e registada a sua massa e foi feita a avaliação da cor com auxílio de um espectrofotómetro antes e após a termociclagem. Após a termociclagem, de modo a estudar a libertação da clorexidina pelos espécimes, os cilindros foram armazenados individualmente em frascos graduados de 5mL e cobertos por saliva artificial, num rácio de 1g/5mL. Estes foram posteriormente incubados a 37ºC e, em intervalos de tempo específicos (2, 4, 7, 24, 48, 72, 144, 168,192,216, 240, 336, 504, 672 horas), foram pipetados 450µL a partir de cada frasco para uma placa de micropoços. As amostras foram de seguida analisadas num espectrofotómetro a 255nm e as absorvâncias foram convertidas em concentrações. Nos mesmos intervalos de tempo, 450µL de saliva artificial foram renovados em cada frasco, de modo a simular a constante renovação salivar.

Foi realizada a análise estatística dos dados dos espécimes incorporados, de forma a verificar a existência de diferenças significativas entre os materiais e entre concentrações. Tendo em conta que os dados não apresentavam uma distribuição normal para as variáveis em estudo, os resultados foram submetidos a testes não paramétricos pelo método de Kruskall-Wallis. Para tal, foi considerado um nível de significância igual a 5%.

Relativamente à variação de massa, observou-se que houve variação estatisticamente significativa antes e após a termociclagem. Nos espécimes de Probase Cold a incorporação do fármaco não causou alterações na variação de massa comparadas com o controlo. Por outro lado, para o Ufi gel Hard e para o Kooliner, a presença de clorexidina conduziu à perda de massa. Esta perda foi mais significativa no Ufi Gel Hard do que no Kooliner. Observou-se ainda que para os grupos controlo em todos os materiais houve um aumento de massa. Considera-se que estes resultados estão associados a alterações físicas já avaliadas em outros estudos como solubilidade e formação de poros na superfície do material que ocorrem quando são sujeitos a alterações térmicas. Acredita-se que estas alterações físicas condicionem a libertação de clorexidina do material e facilitem a entrada de água o que pode justificar estas alterações de massa.

Acerca da estabilidade de cor após a termociclagem, houve variação de cor em todos os materiais e a variação foi diferente entre diferentes resinas. Probase Cold foi o que teve a maior variação de cor seguido do Ufi Gel Hard e do Kooliner. Verificou-se, no entanto, que a presença de clorexidina incorporada não condicionou de forma estatisticamente significativa a mudança de cor em nenhum dos materiais. Tendo em conta que a clorexidina não teve influência significativa, pensa-se que a justificação para os resultados se prenda novamente com os mecanismos já descritos de entrada de água no material.

No que diz respeito à libertação de clorexidina, os resultados mostraram que apenas o Probase Cold teve valores de quantificação de clorexidina superiores ao limite de detecção do método utilizado. Para este material, foi observado que independentemente da concentração de clorexidina, a libertação teve um início rápido e crescente seguido de uma libertação mais lenta e constante até ao final do estudo. Este comportamento está de acordo com a literatura que o justifica com fenómenos iniciais de libertação rápida do fármaco que está à superfície com a posterior libertação lenta do fármaco que se encontra no interior da resina. Observou-se igualmente, que maiores percentagens de clorexidina incorporadas tiveram maiores valores de libertação (μg/mL). Também se verificou que a clorexidina libertada (μg/mL) às 48 horas foi sempre superior a 4.78 μg/mL valor este que de acordo com outros estudos, representa a concentração mínima inibitória para a *candida albicans*. Observou-se ainda que a percentagem máxima de clorexidina libertada (w/w) foi de apenas 1.94% o que significa

que grande parte do fármaco não se libertou durante o período de incubação. Este resultado foi também observado de forma semelhante em outros estudos.

Relativamente às limitações do estudo, a forma cilíndrica dos espécimes não reproduz superfície protética. Surge a necessidade de fazer estudos que avaliem a rugosidade e formação de poros nas resinas acrílicas com clorexidina incorporada após a termociclagem bem como outros estudos microbiológicos e de biocompatibilidade.

O presente estudo conclui que os sitemas de libertação de clorexidina em resinas acrílicas de rebasamento poderão vir a ser uma boa alternativa no tratamento da estomatite protética .

Palavras-chave: Estudos de libertação; Clorexidina; Termociclagem; Resinas acrílicas; Estomatite protética.

ABSTRACT

The use of controlled-release agents has being studied as therapeutic alternatives for the treatment of denture stomatitis.

The main purpose of this study was to evaluate mass variation color stability and drug release of acrylic reline resins loaded with chlorhexidine after thermal aging. Three different materials were evaluated in the present study, Kooliner, Ufi Gel Hard and Probase Cold. For each, one control group and four experimental groups with chlorhexidine (2.5%, 5%, 7.5% and 10% (w/w)) were produced. A total of 45 cylinder-shaped specimens were evaluated. Mass was calculated using a precision scale and color was evaluated using a spectrophotometer. These measurements were made before and after a thermocycling aging procedure of 1000 cycles of thermal fluctuations between 5ºC and 55ºC (20 seconds each bath). After that, the cylinders were stored individually in graduated falcon tubes and covered with saliva at pH=7. The falcons were then placed into an incubator and, at specific time intervals, an aliquot were collected from each falcon and the same amount of artificial saliva was renovated, in order to simulate the constant salivary renovation. The samples were analyzed by UV-spectroscopy and chlorhexidine content was determined. Data were submitted to nonparametric tests according to Mann-Whitney and Kruskall Wallis methods ($p<0.05$).

Results showed that thermocycling caused variationsin mass in all the materials. For Ufigel and Kooliner specimens, chlorhexidine incorporation caused a mass decrease when compared to control. However, clorhexidine incorporation didn't affect mass variation on Probase Cold. It was also observed that thermocycling caused color variation in all the specimens but chlorhexidine incorporation didn't affect the color stability. Color variation was higher in Probase Cold followed by Ufi Gel Hard and Kooliner. after thermocycling, it was perceived that only Probase Cold released chlorhexidine whose quantification was superior to 0.6 μg/mL (minimum detectable capacity of the technique used).

Overall, results indicate that chlorhexidine delivery systems based on acrylic reline resins are a potential approach in the treatment of denture stomatitis.

Keywords: Thermocycling; Release studies; Chlorhexidine; Acrylic resins; Stomatitis

.

INTRODUCTION

Tooth loss is considered one of the most common oral health complications among the elderly population. This unfortunate situation is a result of an accumulation of preventable oral health diseases experienced during a lifetime such as periodontal disease or dental caries (Jones *et al.* 2003; Pisani *et al.* 2011). (Jones et al. 2003)

One of the consequences of tooth loss is the alveolar resorption. This process is continuous and progressive and will cause the desadaptation in local areas of the denture base (Reis *et al.*, 2006; Urban *et al.*, 2007b; Kranjcic *et al.*, 2013, Lyu X, 2016)). Therefore it is very important to make a periodic exam to detect the changes in the adaptation of the denture. (Reis *et al.*, 2006)

A relining procedure is made to overcome the problem of bone resorption, it consists in resurfacing the base of a denture with a new material in order to fill the existing space between the original denture contour and the altered tissue, improving the retention, stability and support of the prosthesis (Ahmad *et al.*, 2009; van Meegen and Kalk, 2011). Some of the materials that can be used to this procedure are the acrylic resins, they consist of polymeric biomaterials and can be classified as chemical, heat or light activated.(Koran III 2002)

The majority of dentures worldwide are fabricated form acrylic resins whose good characteristics like good thermal conductivity, low price and ease of manipulation contribute to its preference. However, acrylic resins still have some handicaps like discoloration and change of physical properties over time (Salloum, 2014) With the prevalence of 45-70%, candida-associated dentures stomatitis is a very common chronic inflammatory disease among denture wearers. Although it's a multifactorial disease (associated with trauma from ill-fitting dentures, bad oral hygiene, reduced saliva secretion, broad spectrum antibiotic disease, immunologic disease among others) the main cause is the presence of *Candida albicans* and biofilm formation. (Redding *et al.* 2009; Rautemaa and Ramage 2011, Lyu X, 2016)) When symptomatic, it's main clinical sign is a diffuse inflammation of the denture-bearing areas (Chandra *et al.* 2001; Amin *et al.* 2009; Cao *et al*. 2010;da Silva *et al.* 2011; Ryalat *et al.* 2011; Salim, Moore, *et al.* 2012a)

Candida albicans is a fungal organism that act as an opportunistic pathogen (Patel *et al.* 2001; Bertolini *et al.* 2014, Lyu X, 2016)) and it is the principal agent contributing to this disease. There is evidence indicating that *Candida* is able to adhere to acrylic resin dentures, namely to polymethylmethacrylate (PMMA) and Polyethilmethylmethacrylate (PEMA) and form biofilms that are a crucial to the pathogeny of stomatitis. (Chandra et al, 2001;Salim Moore, et al 2012; Pereira, 2008, Figueiral, 2015)

Currently, the treatment of denture stomatitis includes the mechanical and chemical control of plaque, the respect of the rest periods of denture wearing and also the use of antimicrobial agents (Silva 2011, Figueiral, 2015). Systemic and topic antifungal treatment is advised but recurrences are frequent (Figueiral, 2015) and one of the problems is that it requires a daily compliance with the appropriate dosage to maintain its efficacy (Salim 2012). An example of this situation is Nystatin that, despite its good efficacy, it requires four applications daily (Moore, et al. 2012; Salim, Moore et al. 2013, Lyu X, 2016)

Fluconazole is a commonly used systemic antifugal due to its low toxicity and mild side effects. However, it doesn't present a good long term efficiency and it has been related to the emergence of resistant strains of C.albicans (Chandra *et al.* 2001;Redding *et al.* 2009; da Silva *et al.* 2011). This way, it is recommended to take other therapeutic/prevention strategies before fluconazole, and leave it to cases of immunodeficiency or to severe cases of stomatitis (Figueiral, 2015).

Many studies have shown that chlorhexidine is able to supress the ability of *candida albicans* to adhere not only to the buccal epithelal cells but also to the surface of the acrylic denures. (Bertolini *et al.* 2014 ; da Silva *et al.* 2011; Peter A. Suci, 2002; Shino B, 2016). Also, it has been proved that chlorhexidine compared to fluconazole, has a higher efficacy against candidal biofilms and, so far, it has not been observed the emergence of resistance strains (Salim, Silikas, *et al.* 2013b). Chlorhexidine has a good substantivity but, in fact, most of the agent is dissolved and removed from the oral cavity thanks to the renovation of saliva and self-cleaning effect of the oral musculature. According to that, the efficacy of the topical agents is limited to a short term action. (Ryalat *et al.* 2011; Salim, Moore, *et al.* 2013a)

To overcome the problem of topical substantivity in the treatment of stomatitis it was suggested the use of drug carriers and controlled-release agents (Riggs *et al*. 2000; Salim, Silikas, *et al.* 2013b). In this method, the antifungal or antimicrobial agent is impregnated into the material in order to be released slowly overtime. This way, it will maintain satisfying therapeutic levels of the medication at the spot of infection (Amin *et al*. 2009) being able to exceed, in some cases, the minimum inhibitory concentration *candida albicans* (MIC) (Gong *et al.* 2007)(Salim, 2013) . One of the advantages of this therapeutic methodology is that the therapeutic effect is achieved with less amount of drug, contributing to a decrease in lateral effects or drug-drug interactions (Bertolini *et al.* 2014). Another benefit is that it is non-compliance-dependent which is especially good to physically or mentally disabled patients (Amin - 2009; Salim 2012; S. J. Wilson and H. J. Wilson 1993;).

Spectroscopy is an easy and reliable method that can measure the release of chlorhexidine from the acrylic resins (Amin *et al.* 2009; Ryalat *et al.* 2011; Salim, Moore, *et al.* 2012a)

However, it is assumed that the physical properties of the acrylic resins will be altered in the presence of the drug's particles. This alteration is caused by de dissolution of the material and porous formation. Furthermore, the porous formation into the material will encourage drug release and increase water uptake (Amin,2009; S. J. Wilson and H. J. Wilson 1993; Hiraishi *et al.* 2008)

Regardless of the big variety of antifungal agents and polymeric systems that have been proposed and studied for oral use as controlled release agents, chlorhexidine is considered one of the best agents to be incorporated in acrylic resins for its better results in terms of releasing and microbial tests (Amin *et al.* 2009; Gong *et al.* 2007; Redding *et al.* 2009; Salim, Moore, *et al.* 2012a).

Evidence shows that the release of chlorhexidine from the acrylic resins depends on its concentration. Also, it has been witnessed that bigger concentrations are released initially supposedly because it represents the release of the drug that is on the surface of the material. Afterwards it starts a second phase where it is observed a slow release of the medicine. That release is a result of a complex process that involves the formation of fluid clusters around the drug molecules Those clusters will interact with the fluid absorption of the acrylic resins (Amin et al 2009; Thaw 1982)

Colour stability is a very important clinical property as it is related to anging or damaging of the materials (Salloum, 2014) Incomplete polimeryzation, diet, oral

hygiene, water sorption and surface rugosity of the material are some of the factors that can affect the amount of colour change in an acrylic resin (S.-K. PARK, 2004; Hatim & Al-Tahho, 2013a) .Colour determination should be accurately measured with spectrophotometers (S.-K. PARK, 2004; Hatim & Al-Tahho, 2013a;)

The international Commission on illumination (CIE) l*a*b system is widely used in dentistry in order to evaluate variations in color in dental materials. The colorimeters are able to measure three parameters of color : l (lightness) a (red/green) and b (yellow/blue). The color difference between two samples is represented as ΔE. If ΔE > 3.3 then the color difference is perceptible by humans. (Hatim & Al-Tahho, 2013b)

Colour stability is a very important clinical property as it is related to anging or damaging of the materials (Salloum, 2014) Incomplete polimeryzation, diet, oral hygiene, water sorption and surface rugosity of the material are some of the factors that can affect the amount of colour change in an acrylic resin (S.-K. PARK, 2004; Hatim & Al-Tahho, 2013a) .Colour determination should be accurately measured with spectrophotometers (S.-K. PARK, 2004; Hatim & Al-Tahho, 2013a;)

The international Commission on illumination (CIE) l*a*b system is widely used in dentistry in order to evaluate variations in color in dental materials. The colorimeters are able to measure three parameters of color : l (lightness) a (red/green) and b (yellow/blue). The color difference between two samples is represented as ΔE. If ΔE > 3.3 then the color difference is perceptible by humans. (Hatim & Al-Tahho, 2013b)

Other authors have studied the release of chlorhexidine from acrylic resins, but they used distilled water as media solution and they didn't study the effects of thermocycling on color and weight variation on the acrylic resins with chlorhexidine incorporation (Hiraishi *et al.* 2008;Salim, Moore, *et al.* 2012a; Bertolini *et al.* 2014). In the present study it was used artificial saliva at pH 7 to simulate the release in the oral cavity. Also, the period of analysis was 28 days, allowing comparisons with other studies (Hiraishi *et al.* 2008; Salim, Moore, *et al*. 2012a; Salim, Silikas, *et al.* 2013b). In addition, four different percentages of chlorhexidine incorporation were evaluated, as opposed to the other studies so far that only studied the release of chlorhexidine in one concentration.

2. OBJECTIVES

The main objective of this study was to evaluate the effect of a thermal aging procedure on the weight and color of three different acrylic reline resins with different percentages of chlorhexidine incorporated, according to the following hypotheses:

H0: Thermocycling doesn't affect the mass of reline resins with incorporated CHX

H1: Thermocycling affects the mass of reline resins with incorporated CHX

H0: Thermocycling doesn't affect the color of reline resins with incorporated CHX.

H1: Thermocycling affects the color of reline resins with incorporated CHX.

Another purpose was to evaluate the release of chlorhexidine from acrylic reline resins after thermocycling, in particularly:

1. The effect of different materials composition (Kooliner, Ufi Gel Hard and Probase Cold) on the drug release;

2. The effect of different chlorhexidine loading percentages (2.5%, 5%, 7.5% and 10%) on the drug release.

3. MATERIALS AND METHODS

In the present study, it was used three auto-polymerizing acrylic resins (Table 3.1) presented in the powder-liquid form. Two of them are direct reline resins: a non-crosslinking material, Kooliner (GC America Inc, Alsip, Illinois, USA) (Figure 3.1a), and a crosslinking material, Ufi Gel Hard (voco GmbH,Cuxhaven, Germany) (Figure 3.1b) and one indirect reline resin, Probase Cold (Ivoclar Vivadent AG, Liechenstein)(Figure 3.1c) that has methylmethacrylate (MMA) as the monomer (Arima et al.,1995 and 1996).

Table 3.1- Materials under evaluation in the study

Product	Manufacturer	Batch number	ratio (g/mL)	Composition	Curing cycle
Kooliner (K)	GC America Inc., Alsip, Illinois, USA	1007201(P) 1008101(L)	1.4/1	P: PEMA L: IBMA	10 minutes 37°C
Ufi Gel Hard (U)	Voco GmbH, Cuxhaven, Germany	1128441(P) 1134070(L)	1.77/1	P: PEMA L: HDMA	7 minutes 37°C
Probase Cold (PC)	Ivoclar Vivadent AG, Liechtenstein	L49853(P) L43809(L)	1.5/1	P: PMMA L: MMA	15 minutes 40°C 2-4 bar

P - Powder, L - Liquid, PEMA - polyethyl methacrylate, IBMA – isobutyl methacrylate, HDMA - hexanediol dimethacrylate PMMA - polymethyl methacrylate, MMA - methyl methacrylate.

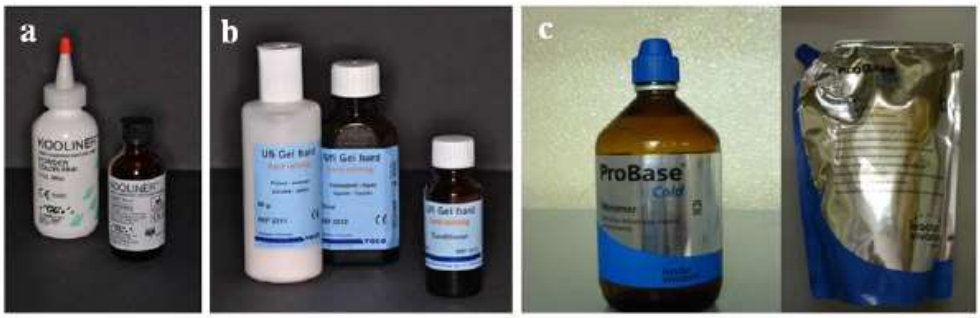

Figure 3.1 Materials under evaluation in the study : a) Kooliner; b) Ufi Gel Hard; c) Probase Cold

3.1 Preparation Of The Specimens

The acrylic resins were manipulated according to the manufacturer's instructions (Table 3.1). The powder was weighed using a precision balance (Mettler Toledo) and the liquid was measured using a pipette. On the experimental specimens, chlorhexidine diacetate monohydrate (CHX) (Panreac Applichem, Darmstadt, Germany) (Figure 3.2a) was incorporated at a proportion of 2.5%, 5%, 7.5% and 10% of the acrylic resin's powder weight (w/w) and mixed using a mortar and pestle for homogenization (figure 3.2b)

Five groups of specimens were produced for each material (one control group without CHX and four experimental groups using the CHX percentages mentioned), that resulted in fifteen specimens per material (n=15), three of each group (Table 3.2).

The cylinder-shaped specimens with the size of 12mm height and 6m diameter (Figure 3.2c) were produced using stainless steel molds (Figure 3.2b). A total of 45 specimens were prepared for this study.

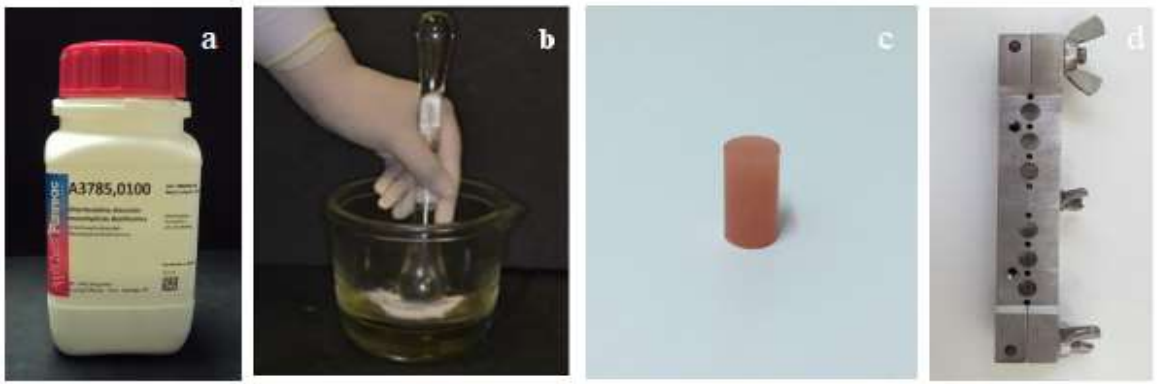

Figure 3.2 Preparation of the specimens: a) Package of the Chlorhexidine diacetate monohydrate: b) Incorporation and homogenization of the CHX; c) Cylinder-shaped specimens d) Cylinder-shape mold.

In each preparation of Kooliner and Ufi Gel specimens, the materials dough was poured into the cylinder-shaped molds, maintained at the temperature of 37±2ºC, during the recommended polymerization time (Table 3.1) in order to simulate the intraoral polymerization of direct reline resins. Polymerization of the indirect reline resin was carried out in a pressure device (Ivomat,Ivoclar Vivadent, Liechenstein)(Figure 3.3) at recommended time, temperature and pressure (Table 3.1)

Figure 3.3 Ivomat pressure device

3.2 Thermocycling Aging Process

All the specimens (n=45) were exposed to a thermocycling aging procedure of 1000 cycles of thermal fluctuations between 5ºC and 55ºC (20 seconds each bath), with 5 seconds of dwell time, in a specific machine (Refri 200-E, Aralab, Cascais, Portugal) (Figure 3.4).

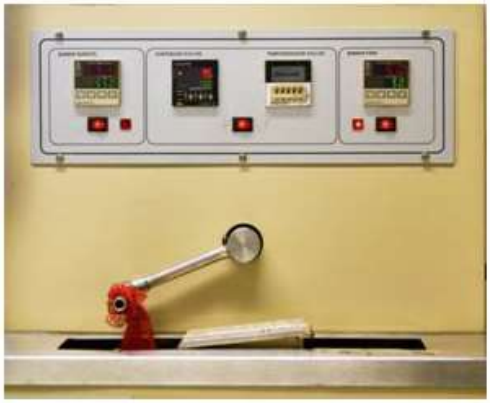

Figure 3.4 - Thermocycling equipment

3.3. Mass Determination

It was measured all the specimen's mass (n=45) with a precision scale, before and after the thermocycling. Specimens were divided in each material in "with clorhexidine"- meaning all the specimens with some concentration of clorhexidine and "without clorhexidine" wich included the control group.

3.4 Color Determination

Color was determinated using a Easyshade spectrophotometer (Fig3.5) inside of a dark box (fig 3.6) and 3 evaluations were made at the bottom and the top of each specimen. The specimens were measured with a spectrophotometer, using the CIE L*a*b* system, at three specific times : before thermocycling aging ; after thermocycling aging and after the in vitro relase study

Figure 3.5 – Vita Easyshade equipment

Figure 3.6 – Dark box

3.5 ANALITICAL METHODOLOGY

3.5.1 Standard stock and releasing solutions

To prepare a standard stock solution of 1000 ug/ml it was dissolved approximately 10mg of CHX into 10 ml of deionized water. This solution was kept out of light and at room temperature. On each new measurement of CHX, a series of dilutions of the stanrd stock solution were prepared (125; 62,5; 31,25; 15,62; 7,81; 3,905 μg/mL)

In this present study, the selected releasing solution was artificial saliva at pH=7 (Figure 3.7), to preview how the CHX would be released in the oral cavity. The

artificial saliva was prepared according to a Faculty of Pharmacy University of Lisbon formula, courtesy of PhD student Joana Marto:

Boiling 50mL (F12-ED Refrigerated/Heating Circulator) of phosphate buffer pH=7.0 (Anhydride disodium phosphate, Monosodium phosphate anhudride and Deionized water) at 60ºC. Then sprinkled 0.05g of xanthan gum into boiling buffer and stirring until total of xanthan gum was dissolved.

Dissolving 0.04g of Calcium chloride dihydrat (EW-N/EG-N balance) 0.08g of Sodium chloride and 0.08g of Potassium chloride in solution 1 and stirring until total of materials were dissolved.

Dissolving 15g of Propylene glycol in solution 2 and stirring until total of Propylene glycol was dissolved.

Pouring the solution 3 into graduated beaker and complete the solution with phosphate buffer pH=7.0 to 100mL

This solution was kept out of light, at room temperature.

.

Figure 3.7 - Artificial Saliva at pH 7

3.5.2 Analytical Technique

The absorbance of each solution was measured in a microplate reader (FLUOstar Omega- BMG LABTECH) (Figure 3.8) and the absorbance values were obtained using an Ultraviolet-Visible absorbance Spectra detection mode, with a wavelength of 255nm, as recommended by other authors (Anusavice et al. 2006). The measurements were performed at room temperature of 25ºC.

The CHX release concentrations were determined based on the linear calibration methodology, after subtracting the average of control's absorbance at the corresponding time interval.

Figure 3.8 – Microplate reader

3.6 IN VITRO RELEASE STUDIES

A preliminary pilot study was conducted in order to optimize further experimental protocols.

With the aim to study the release of CHX from the specimens, the cylinders were stored individually in graduated falcon tubes of 5mL and covered with saliva pH=7, with a ration of 1g/5,mL (figure 3.9a) The Falcons were then placed into an incubator at 37ºC (Memmert), with constant gentle shaking (300rpm) (Figure 3.9b). At specific time intervals (1,4,24,48,72,144,168,192,216,240,336,504,672 hours)(Table 3.2), and after the falcons were agitatd in a mixer (VELP Scientifica, Vortex), 450μLwere pipetted from each falcon to a polystyrene flat bottom microplate wells (96-well microplates) (150μL were pipetted to each well). At the same time intervals, 450μL of artificial saliva at pH=7 were renovated in each falcon, in order to simulate the constant salivar renovation. The samples were analyzed as described above.

Figure 3.8 - Incubation of the speciens in graduated falcon tubes with saliva at ph=7

Figure 3.9 - Incubator at 37 ºC under constant gentle shaking

3.7. STATISTICAL ANALYSIS

Data were statistically analyzed using SPSS Statistics 20 (SPSS Inc., Chicago, IL, USA).

Descriptive statistics of mass, color variation and release data was carried out.

Since data did not follow a normal distribution for the studied variables (verified by Kolmogorov-Smirnov normality tests), the results were submitted to the nonparametric tests according to the Kruskall Wallis method followed by multiple comparisons using Mann-Whitney tests with Bonferroni correction to determine whether there were specific significant differences among materials and groups.

In all statistical tests, it was considered the 5% level of significance ($p<0.05$)..

Table 3.2 – Schematization of distribution of the specimens

Material	Group	CHX incorporation	Releasing solution	Time intervals
Kooliner	1 control group	Without CHX (n=3)	Artificial Saliva pH= 7	1,4,24,48,72,144,168,192,216,240,336,504,672 hours
	4 experimental groups	With CHX 2.5% w/w (n=3)		
		With CHX 5% w/w (n=3)		
		With CHX 5% w/w (n=3)		
		With CHX 7.5% w/w (n=3)		
		With CHX 10% w/w		
Ufi Gel Hard	1 control group	Without CHX (n=3)		
	4 experimental groups	With CHX 2.5% w/w (n=3)		
		With CHX 5% w/w (n=3)		
		With CHX 5% w/w (n=3)		
		With CHX 7.5% w/w (n=3)		
		With CHX 10% w/w		
Probase Cold	1 control group	Without CHX (n=3)		
	4 experimental groups	With CHX 2.5% w/w (n=3)		
		With CHX 5% w/w (n=3)		
		With CHX 5% w/w (n=3)		
		With CHX 7.5% w/w (n=3)		
		With CHX 10% w/w		

4. RESULTS

4.1 The effect of thermal aging in mass variation

Thermocycling caused mass variation in all the different materials (p<0.001).

Kooliner specimens with CHX had a mass decrease of 0,33557±0.2% compared to specimens without CHX (p=0.04). Ufi Gel Hard specimens with CHX had a mass decrease of 0,81435±0.4% when compared to specimens without CHX (p=0.018).

Probase Cold had had no significant differences in mass variation (p= 0.365).

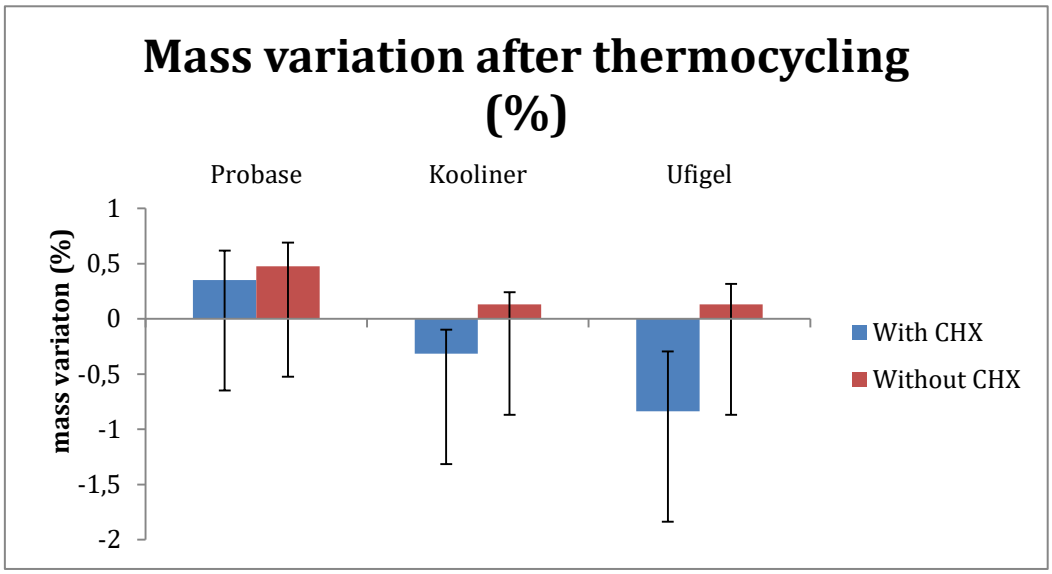

Figure 4.1 Mass variation (%) between different materials and CHX loading

4.2 The effect of Thermal aging on color variation

Thermocycling caused color variation in all the different materials ($p<0.001$)

After thermocycling, specimens were considered having higher color variation in Probase (ΔE= 8,176424) than Ufi Gel (ΔE= 7,045814) and kooliner (ΔE= 4,34457) . Specimens of Ufi Gel Hard had higher values than Kooliner ($p<0.001$)

In each material, CHX incorporation didn't lead to significant differences in ΔE ($p=0.631$)

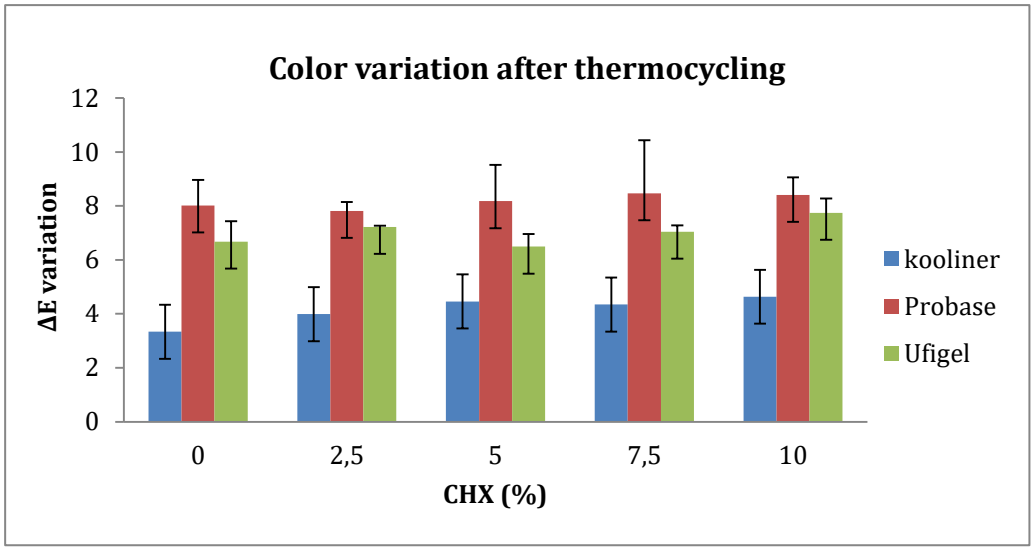

Figure 4.2 Color variation (ΔE) between different materials and CHX loading (2.5%, 5%, 7.5%,10%)

4.3. CHX release

4.3.1 CHX quantification

A linear relationship between CHX concentrations and the absorbance peak areas was established at 255 nm The analytical method showed good linearity (Figure 4.1).

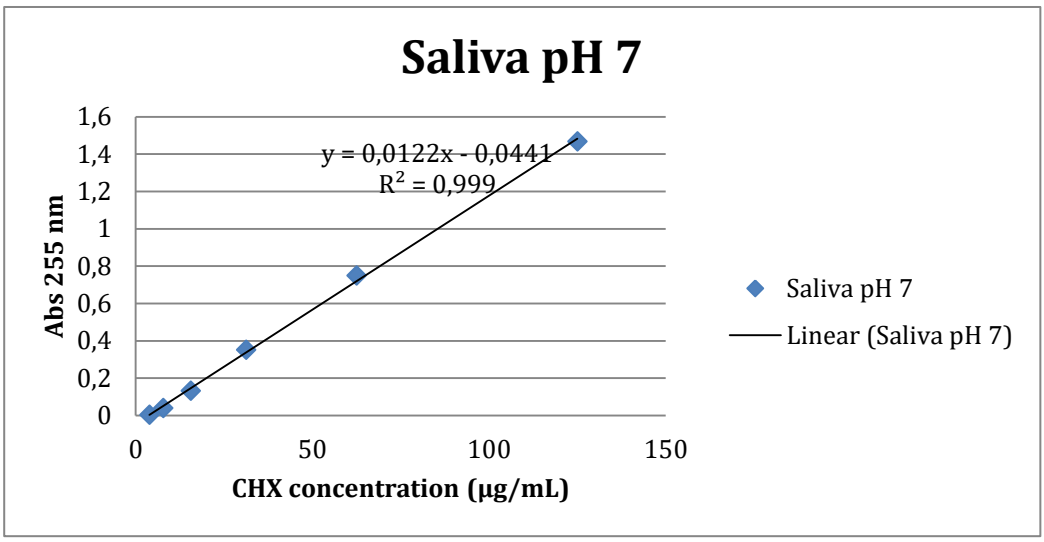

Figure 4.3 – Linear relationship between CHX concentrations and the absorbance peak areas for artificial saliva at pH 7

4.3.2. The effect of different materials on the drug release

Specimens of three different materials (Kooliner, Ufi Gel Hard and Probase Cold) were evaluated in the present study, with CHX 2.5%, 5%, 7.5% and 10% (*w/w*), after thermocycling.

In K and U, the CHX quantified from all specimens was inferior to 0.6 µg/mL that was the minimum detectable capacity of the technique used in the present study.

Considering Probase Cold specimens, it was observed a high rate of release until 336 h followed by a slower and steadier release until the end of the study (28 days) (Figure 4.2).

For CHX 2.5%, 14.493 µg/mL from Probase Cold were released until 48 hours of incubation. For CHX 5% , 22.832 µg/mL were released from Probase Cold were released until 48 hours . For CHX 7.5%, 35.895 µg/mL were released from Probase Cold were released until 48 hours. For CHX 10 %, 33.619 µg/mL were released from Probase Cold until 48 hours

All the comulative values were higherthan MIC described by Salim (Salim et al. 2013a)

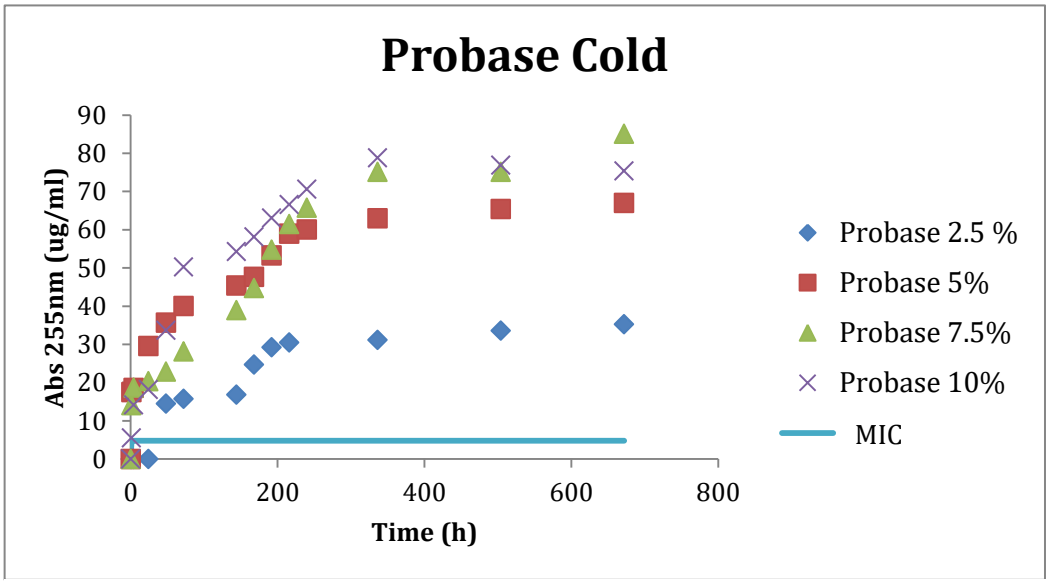

Figure 4.4 – Line diagram with the cumulative CHX concentration (µg/mL) versus time (hours) for Probase

The maximum cumulative release (µg/mL) from Probase Cold during the study, is shown in Table 4.1. The results show that the maximum cumulative release is only 0.195%, meaning that only a small amount of initial loaded CHX is liberated from the specimens to the artificial saliva.

Table 4.1 Maximum cumulative release of chlorhexidine for Probase with CHX 2.5%, 5%, 7.5%, 10%.

Material	Group (%)	Maximum Cumulative Release (µg/mL)	t(h)	% (w/w)
	2.5	31,12803	336	0,032886
Probase Cold	5	62,98447	336	0,043487
	7.5	75,05869	336	0,050299
	10	78,74153	336	0,194504

DISCUSSION

Several studies have investigated the use of polymers as drug delivery systems for slow release of antifungal drugs in order to treat oral infections (Li *et al.* 2009b; Salim, Satterthwaite, *et al.* 2012b).

Early Studies have mentioned that chlorhexidine is superior to other antifungal agents both on releasing and microbiological tests when compared to other antifungal drugs. (Amin *et al.* 2009; Redding *et al.* 2009; Salim, Moore, *et al.* 2012a; Salim, Moore, *et al.* 2013a; Salim, Silikas, *et al.* 2013b) However, there weren't found many studies regarding the evaluation of physical changes over time of these acrylic resins with antimicrobial agents incorporated. (Goiato, 2009; Landayan,, 2014; S.-K. PARK, 2004) (Sousa 2014) () (2014)

The aim of the present study was to evaluate mass and color variation of three different reline acrylic resins with different drug loading after 1000 cycles of thermocycling between 5ºC and 55ºC. This method was selected because it is believed to simulate 6 weeks of thermal variation in the oral cavity (Gale and Darvell, 1999) caused by routine eating, drinking and breathing (Palmer *et al.*, 1992). In addition it was tested the release capability of those resins after the aging process.

In terms of mass variation, it was hypothesized that thermocycling wouldn't affect the mass of acrylic reline resins with incorporated chlorhexidine. However, the results have shown that there were statistically significant mass variations after thermocycling in the different materials. In Ufi Gel Hard and Kooliner the incorporation of drug caused differences in the mass variation. There was observed a mass gain in the control specimens and a mass decrease in the specimens with chlorhexidine.

In Probase Cold there was a general mass increase after thermocycling in all the specimens with no differences between the controls and the specimens with incorporated chlorhexidine. According to that, the null hypothesis was accepted. Probase mass gains during thermocycling might have been caused by water absorption from the specimens. In fact, studies have suggested that thermal fluctuations can cause surface stresses due to the high thermal gradients near the surface. Those mechanical stresses can directly induce crack propagation, porous

formation and increase in solubility that will lead to water absorption (Bettencourt *et al.* 2010, Goiato, *et al*. 2009a and 2009b,Landayan,*et al*. 2014, S.-K. PARK, 2004, Sousa, 2014). On the other hand, it is believed that the mass loss observed in Ufi Gel Hard and Kooliner was caused by the release of chlorhexidine. However, if there was any water absorption from those resins it was not enough to compensate significantly the weight loss caused by the release of chlorhexidine. This makes sense if we consider that the molecular weight of chlorhexidine is much higher than water.

About color variation, it was hypothesized that thermocycling wouldn't affect color stability in the acrylic resins with chlorhexidine incorporated. The results prove that there were differences in color variation after the thermocycling in all specimens. Also, it was observed that there were no differences between specimens with and without chlorhexidine. And this way, the null hypothesis was accepted.

Probase Cold was the material that had higher values of color variation followed by Ufi Gel Hard and Kooliner. This is in agreement with other studies that also found color variations in acrylic resins after thermocycling. However they didn't test the effect of drug loading on that variation as we did in our study. The reason for color change might be related to intrinsic properties of each material, as well as dissolution of plasticizers and colorants. Also, the physical changes caused by thermocycling and water sorption could justify that color instability. In fact it is believed that water absorption and surface roughness affects color stability (Salloum, 2014) Color variation is also related to aging or damaging of the material (Goiato, 2009)

After thermocycling, the release study showed that in Kooliner and Ufi Gel specimens the chlorhexidine quantified from all specimens was inferior to 0.6 μg/ml (minimum detectable capacity of the technique used in the present study), Probase Cold was the only material that had release values superior than those.

Probase Cold had a high rate of initial release followed by a slower and steadier release that continued until the end of the study period. This agrees with earlier studies that explain that chlorhexidine release is controlled by a concentration dependent diffusion process (Amin *et al.* 2009, Anusavice *et al.* 2006; Bertolini *et al.* 2014; Gong *et al.* 2007; Hiraishi *et al.* 2008;; Li *et al.* 2009; Marcelino 2015; Ryalat

et al. 2011; Salim, Moore, *et al.* 2012a;,).It is believed that initially the drug that is on the surface of the material is released rapidly. Afterwards it starts a second phase where there is a slow release of the medicine that is a result of a complex process involving the formation of fluid clusters around the drug molecules that will interact with the fluid absorption of the acrylic resins (Thaw 1982, Amin et al 2009)

The maximum cumulative release of the present study was 0,194% for PC. This signifies that a very small amount of the initial chlorhexidine incorporated was released to the artificial saliva. This percentage is lower than what was observed in other studies (Patel *et al.*2001; Salim, Moore, *et al.* 2012a) and the reason for that is probably because some part of the chlorhexidine might have been released during the thermocycling procedure which means that this process is not adequate to simulate aging in release studies.

In the present study, it was used artificial saliva at pH 7 to simulate oral cavity conditions because properties like viscosity can have an influence in the results. Most of the studies that were found used distilled water as media solution (Hiraishi *et al.* 2008; Salim, Moore, *et al.* 2012a; Bertolini *et al.* 2014). The total length of the study (28 days) was selected in order to compare with the existing investigations of the same length.

In contrast to the majority of the other studies that only measured the release of chlorhexidine in one concentration, in the present study four different chlorhexidine concentrations were evaluated in order to find the minimum concentration that is effective against *C. albicans* and at the same time preventing an allergic reaction by the host.

It is believed that chlorhexidine was released during thermocycling from Ufi Gel Hard and Kooliner because another study have tested the release capability of those materials with similar conditions and same chlorhexidine loading but without the effect of thermocycling and there was observed the release of much higher values of chlorhexidine (Neuza, 2015). The cause of drug release during the aging process may be related to the composition of the acrylic resins. Polyethylmethacrylate based materials like Ufi Gel Hard and Kooliner are known for their anomalous water uptake

(Riggs *et al.* 2000; Patel *et al.* 2001; Salim, Moore, *et al.* 2012a; Salim, Satterthwaite, *et al.* 2012b) that will cause a superior drug release compared to the materials with methylmethacrylate like Probase ColdC (Patel et al. 2001). Compared to other drugs such as Fluconazole, chlorhexidine has higher solubility (Salim, Satterthwaite, et al 2012). Moreover, in Polymethylmethacrylate materials, after the droplet expansion it is observed the formation of cracks that will lead to a release of the drug (Addy and Thaw 1982; Riggs *et al.* 2000; Patel *et al.* 2001; Amin *et al.* 2009; Salim, Satterthwaite, *et al.* 2012b).

According to the findings of the present study, it is suggested to use Probase Cold chlorhexidine 2.5% because that percentage of chlorhexidine was enough to maintain minimum inhibitory drug releases for *candida albicans* (Salim 2013). for a long time and it will be less likely to cause allergic reactions to the host (Amin *et al.* 2009; Bertolini *et al.* 2014). In addition, Probase cold was the material whose release capability wasn't affected seriously when exposed to the type of thermal variations that will be observed in the oral cavity. Even though it was the material whose color variation was the most perceived clinically, his therapeutic effect for long periods surpasses that disadvantage.

In respect of study limitations the size of the specimens used in this *in vitro* study is very different from the denture surface. In fact, drug release is related to surface area as smaller areas will expose less drug particles to the saliva (Salim, Moore, *et al.* 2012a), this can be compensated by the higher number of surfaces releasing chlorhexidine in the cylinders while in the denture would only release from one surface. The use of Thermocycling aging is not the best method to apply when a posterior release study is going to be performed because it causes drug releases that can't be quantified during the aging process. The cylinder shape was not the best option to evaluate color variation as it only gives 2 good surfaces to read. A suggestion would be to make the specimens in a shape that allows more different readings but also that is more similar to the denture surface.

In future studies, it will be important to study the surface roughness of the specimens as well as other mechanical characteristics right after thermocycling and also after the release study. This way, it would be easier to find the causes of weight

and color variation between materials and different drug loads. Also, it is important to associate microbiological and biocompatibility tests.

To sum up it is difficult to predict the duration and the physical properties of delivery systems based on acrylic reline resins when incorporated with chlorhexidine. Clinical studies should be performed in order to expose the materials to the complexity of the oral cavity and, this way, guide the implementation of this system in clinical practice.

CONCLUSION

Within the limitations of this study, the main conclusions are:

- Thermocycling induced significant changes in all the specimens in terms of weight, color stability and drug release capacity. Probase Cold was the only material that had appreciable release values after the aging process.
- The incorporation of CHX into reline acrylic resins affects drug release and weight variation but it doesn't affect significantly the color variationafter thermocycling
- To all the Probase Cold specimens with added CHX it was observed a fast initial release followed by a slower and steadier relase.
- In Probase Cold, maximum CHX release was 0.6% meaning that only a small amount of initial loaded chlorhexidine was liberated.
- All the drug loading specimens in Probase had cumulative concentrations superior than MIC values against *C. albicans* isolates.

7. REFERENCES

Addy, M. and Thaw, M., 1982. In vitro studies into the release of chlorhexidine acetate, prednisolone sodium phosphate, and prednisolone alcohol from cold cure denture base acrylic. *Journal of Biomedical Materials Research Part B: Applied Biomaterials*, 16(2), pp.145–157.

Amin, W.M. *et al*., 2009. A new form of intraoral delivery of antifungal drugs for the treatment of denture-induced oral candidosis. *European journal of dentistry*, 3(4), pp.257–266.

Anusavice, K.J., Zhang, N.-Z. and Shen, C., 2006. Controlled release of chlorhexidine from UDMA-TEGDMA resin. *Journal of Dental Research*, 85(10), pp.950–954.

Arima, T., Murata, H. and Hamada, T., 1995. Properties of highly cross-linked autopolymerizing reline acrylic resins. *The Journal of prosthetic dentistry*, 73(1), pp.55–59.

Bertolini, M.M. *et al.*, 2014. Resins-based denture soft lining materials modified by chlorhexidine salt incorporation: An in vitro analysis of antifungal activity, drug release and hardness. *Dental Materials*, 30(8), pp.793–798.

Bettencourt, A 2010. Biodegradation of acrylic based resins : A review. Dental materials, e171-e180.

Cao, Z. *et al*., 2010. Rechargeable Infection-responsive Antifungal Denture Materials. *Journal of Dental Research*, 89(12), pp.1517–1521.

Chandra, J. *et al*., 2001. Antifungal Resistance of Candidal Biofilms Formed on Denture Acrylic in vitro. *Journal of Dental Research*, 80(3), pp.903–908.

da Silva, P.M.B. *et al*., 2011. Microscopical analysis of Candida albicans biofilms on heat-polymerised acrylic resin after chlorhexidine gluconate and sodium hypochlorite treatments. *Mycoses*, 54(6), pp.e712–e717.

El-Hadary, A., & Drummond, J. L. (2000). Comparative study of water sorption, solubility, and tensile bond strength of. *THE JOURNAL OF PROSTHETIC DENTISTRY*.

Figueiral, F. P.-L.-M. (2015). Effect of Denture-Related Stomatitis Fluconazole Treatment on Oral Candida albicans Susceptibility Profile and Genotypic Variability. *Open Dent J*, 46-51.

Gong, K. *et al*., 2007. Controlled release of chlorhexidine diacetate from a porous methacrylate system: Supercritical fluid assisted foaming and impregnation. *Journal of Pharmaceutical Sciences*, 96(8), pp.2048–2056

Hiraishi, N. *et al*., 2008. Chlorhexidine release and water sorption characteristics of chlorhexidine-incorporated hydrophobic/hydrophilic resins. *Dental Materials*, 24(10), pp.1391–1399.

Hatim, N. A., & Al-Tahho, O. Z. (2013). Comparative Evaluation of Color Change Between Two Types of Acrylic Resin and Flexible Resin After Thermo Cycling. An In Vitro Study. *The Journal of Indian Prosthodontic Society*, 327-337.

Hatim, N. A., & Al-Tahho, O. Z. (2013). Comparative Evaluation of Color Change Between Two Types of Acrylic Resin and Flexible Resin After Thermo Cycling. An In Vitro Study. *The Journal of Indian Prosthodontic Society*, 327-337.

Jordi Izzard Andaya Landayan, e. a. (2014). Effect of aging on tear strength and cytotoxicity of soft denture lining materials; in vitro. *Adv Prosthodont*.

Li, J. *et al*., 2009. In vitro drug release study of methacrylate polymer blend system: effect of polymer blend composition, drug loading and solubilizing surfactants on drug release. *Journal of Materials Science: Materials in Medicine*, 21(2), pp.583–588.

Lyu X, Z. C. (2016). Efficacy of nystatin for the treatment of oral candidiasis: a systematic review and meta-analysis. *Drug Des Devel Ther*.

Marcelino, N. Effect of Chlorhexidine Incorporation on Acrylic Reline Resins-Release Studies. Dissertação (Mestrado integrado em Medicina Dentária) Faculdade de Medicina Dentária da universidade de Lisboa; 2015

Marcelo Coelho Goiato, B. C. (2009). EFFECTS OF THERMOCYCLING ON MECHANICAL PROPERTIES OF SOFT LINING MATERIALS. *Acta Odontol. Latinoam*, 227-232.

Marcelo Coelho Goiato, R. M.-A. (2009). EVALUATION OF HARDNESS AND COLOR STABILITY IN THE SOFT LINING MATERIALS AFTER THERMOCYCLING AND CHEMICAL POLISHING. *Acta Odontol. Latinoam.*, 63-68.

Patel, M.P. *et al*., 2001. A polymeric system for the intra-oral delivery of an anti-fungal agent. *Biomaterials*, 22(17), pp.2319–2324. Rautemaa, R. and Ramage, G., 2011. Oral candidosis – Clinical challenges of a biofilm disease. *Critical Reviews in Microbiology*, 37(4), pp.328–336.

Redding, S. *et al*., 2009. Inhibition of Candida albicans biofilm formation on denture material. *YMOE*, 107(5), pp.669–672.

Paulo Maurı´cio Batista da Silva, 1. J. (2011). Microscopical analysis of Candida albicans biofilms on heat-polymerised acrylic resin after chlorhexidine gluconate and sodium hypochlorite treatments. *Mycoses*, 54-58.

Pereira-Cenci T, D. B. (2008). Development of Candida-associated denture stomatitis: new insights. *J Appl Oral Sci*, 86-94.

Peter A. Suci, B. J. (2002). Action of Chlorhexidine Digluconate against Yeast and Filamentous Forms in an Early-Stage Candida albicans Biofilm. *Antimicrob Agents Chemother*, 3522–3531.

Polyzois GL1, Y. S. (1999). Color stability of visible light-cured, hard direct denture reliners: an in vitro investigation. *Int J Prosthodont*, 140-6.

Riggs, P.D. *et al*., 1999. The water uptake of poly(tetrahydrofurfuryl methacrylate). *Biomaterials*, 20(5), pp.435–441.

Riggs, P.D., Braden, M. and Patel, M., 2000. Chlorhexidine release from room temperature polymerising methacrylate systems. *Biomaterials*, 21(4), pp.345–351.

Ronanki S, K. S. (2016). Efficacy of commercially available chlorhexidine mouthrinses against specific oral microflora. *Indian J Dent Res*, 48-53.Ryalat, S., Darwish, R. and Amin, 2011. New form of administering chlorhexidine for treatment of denture-induced stomatitis. *Therapeutics and Clinical Risk Management p.219*

Salim, N., Moore, C., *et al*., 2012a. Fungicidal amounts of antifungals are released from impregnated denture lining material for up to 28 days. *Journal of Dentistry*, 40(6), pp.506–512.

Salim, N., Moore, C., *et al*., 2013a. Chlorhexidine is a highly effective topical broadspectrum agent against Candida spp. *International journal of antimicrobial agents*, 41(1), pp.65–69.

Salim, N., Satterthwaite, J.D., *et al*., 2012b. Impregnation with antimicrobials challenge bonding properties and water sorption behaviour of an acrylic liner. *Journal of Dentistry*, 40(8), pp.693–699.

Salim, N., Silikas, N., *et al*., 2013b. Chlorhexidine-impregnated PEM/THFM polymer exhibits superior activity to fluconazole-impregnated polymer against Candida albicans biofilm formation. *International journal of antimicrobial agents*, 41(2), pp.193–196.

Salloum, A. M. (2014). Effect of 5.25 % Sodium Hypochlorite on Color Stability of Acrylic and Silicone Based Soft Liners and a Denture Base Acrylic Resin. *J Indian Prosthodont Soc*, 179–186.

S.-K. PARK, Y.-K. L.-S.-W. (2004). Changes in properties of short-term-use soft liners after thermocycling. *Journal of Oral Rehabilitation*, 717–724

Wala M. Amin, a. M.-A.-T. (2009). A New Form of Intraoral Delivery of Antifungal

APPENDICES

Appendice 1- List of Tables

APPENDIX 2 – LIST OF FIGURES

APPENDIX 3 – LIST OF ABBREVIATIONS

1.6- HDMA	1.6-hexanedioldimethacrylate
C.*albicans*	*Candida albicans*
CHX	chlorhexidine
h	Hours
IBMA	Isobutylmethacrylate
K	Kooliner
L	Liquid
M	Mean
m	Mass
MIC	Minimum inhibitory concentration
MMA	Methylmethacrylate
P	Powder
PEMA	Polyethylmethacrylate
PMMA	Polymethylmethacrylate
SD	Standard deviation

APPENDIX 4 – EXPERIMENTAL DATA

Mass variation (%)

		CHX % (w/w)				
Material		0	2,5	5	7,5	10
Probase Cold	M	0,477627	0,128733	0,304105	0,401405	0,441826
	SD	0,313694	0,741256	0,951704	0,391605	0,180004
Ufi Gel Hard	M	0,132661	-0,22727	-3,84421	-1,2987	-0,37244
	SD	0,183151	0,553177	0,603677	0,266592	0,527019
Kooliner	M	0,131199	-0,52274	-0,73973	-0,10596	-0,07853
	SD	0,109487	0,152848	0,228223	0,599137	0,205076

Color Variation

		CHX % (w/w)				
Material		0	2,5	5	7,5	10
Probase Cold	M	8,018767	7,816454	8,176424	8,469745	8,408993
	SD	0,946694	0,329665	1,344761	1,965721	0,646104
Ufi Gel Hard	M	6,677866	7,223732	6,49068	7,045814	7,74022
	SD	0,754326	0,045325	0,467175	0,228654	0,530888
Kooliner	M	3,336215	3,986597	4,458546	4,34457	4,633626
	SD	0,688525	0,041243	1,349876	1,12548	0,420405

Release study

Probase Cold 2.5%				
Time intervals (hours)	M(comulative concentration)	SD(Comulative concentration)	M(CHX %released)	SD(CHX %released)
0	0	0	0	0
1	0	0	0	0
4	0	0	0	0
24	12.49268	0.054843	0.042366	0.069816
48	14.49262	0.153142	0.023565	0.051681
72	15.70131	0.061365	0.035062	0.092262
144	16.82454	0.035528	0.026846	0.065218
168	24.68697	0.05847	0.0.39549	0.081469
192	29.23516	0.077853	0.021652	0.049258
216	30.4468	0.063644	0.0216199	0.051816
336	30.9254	0.072923	0.0261881	0.0218969
504	31.12803	0.087821	0.05656	0.091285
672	33.55959	0.065986	0.050278	0.851836

Probase Cold 5%				
Time intervals (hours)	M(comulative concentration)	SD(Comulative concentration)	M(CHX %released)	SD(CHX %released)
0	0	0	0.044883	0.0596816
1	17.49262	0.08414	0.083044	0.0715481
4	18.58101	0.106275	0.124145	0.056262
24	29.51054	0.093798	0.143609	0.037218
48	35.6946	0.1014	0.155751	0.025869
72	40.01578	0.145438	0.164431	0.057938
144	45.3652	0.060514	0.174309	0.067816
168	47.66471	0.069383	0.194504	0.8458969
192	53.21193	0.098097	0.189813	0.063785
216	58.94509	0.086797	0.186083	0.051836
336	60.06124	0.08789	0.044883	0.031451
504	62.98447	0.096146	0.083045	0.0184851
672	65.37444	0.08896	0.124145	0.0486151

Probase Cold 7.5%				
Time intervals (hours)	M(comulative concentration)	SD(Comulative concentration)	M(CHX %released)	SD (CHX % released)
0	0	0	0	0
1	18.65991	0.158173	0.176516198	0.0545481
4	20.35455	0.044721	0.186884641	0.065262
24	22.83224	0.076164	0.296812024	0.038418
48	28.15926	0.07535	0.402472028	0.0632869
72	38.90505	0.182313	0.479403675	0.087838
144	44.70762	0.100316	0.535196725	0.0987816
168	54.76601	0.042287	0.592859873	0.789695
192	61.43249	0.117726	0.601186155	0.0987785
216	65.70508	0.061996	0.621861814	0.098366
336	75.05869	0.087097	0.633487251	0.0978451
504	75.1279	0.038772	0.657525129	0.0975884
672	85.02716	0.092054	0.673529431	0.04526151

Probase Cold 10%				
Time intervals (hours)	M(comulative concentration)	SD(Comulative concentration)	M(CHX %released)	SD(CHX %released)
0	0	0	0	0
1	5.524365007	0.067015	0.186884641	0.071481
4	14.14065826	0.052922	0.296812024	0.046262
24	18.16993777	0.065864	0.402472028	0.012218
48	33.61890654	0.024396	0.479403675	0.035869
72	50.25785615	0.103822	0.535196725	0.034238
144	54.25675626	0.016851	0.592859873	0.055216
168	58.13757875	0.034493	0.6113151316	0.2358969
192	63.05333277	0.053648	0.625565186	0.0342785
216	66.56722166	0.010837	0.633487251	0.065836
336	70.56621225	0.052151	0.657525129	0.052451
504	78.74153101	0.020474	0.673529431	0.05254851
672	76.84272257	0.073326	0.276516198	0.0656151